New Antimitotic Agents with Activity in Multi-Drug-Resistant Cell Lines and in Vivo Efficacy in Murine Tumor Models

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During a screen for compounds that could inhibit cell proliferation, a series of new tubulin-binding compounds was identified with the discovery of oxadiazoline **1** (A-105972). This compound showed good cytotoxic activity against non-multi-drug-resistant and multi-drug-resistant cancer cell lines, but its utility in vivo was limited by a short half-life. Medicinal chemistry efforts led to the discovery of indolyloxazoline **22g** (A-259745), which maintained all of the in vitro activity seen with oxadiazoline **1**, but also demonstrated a better pharmacokinetic profile, and dose-dependent in vivo activity. Over a 28 day study, indolyloxazoline **22g** increased the life span of tumor-implanted mice by up to a factor of 3 upon oral dosing. This compound, and others of its structural class, may prove to be useful in the development of new chemotherapeutic agents to treat human cancers.

Cancer is the second leading cause of death in industrialized nations. Cancer chemotherapy commonly involves the use of cytotoxic agents that destroy rapidly dividing cells. Within the past decade, advances in our understanding of the cell cycle have presented new targets that may allow for the development of more selective chemotherapeutic agents—agents that target only cancer cells. Despite this progress, cytotoxic agents will remain a mainstay in cancer chemotherapy for the near future.

Antimitotic agents constitute a major class of cytotoxic drugs and have been the subject of several reviews.[1−5] At the molecular level, these agents commonly interfere with the dynamics of tubulin polymerization and depolymerization. Tubulin is a heterodimer of two closely related and tightly linked globular polypeptides called α-tubulin and *β*-tubulin. Tubulin molecules polymerize to form long stiff microtubules that extend throughout the cytoplasm and govern the location of membrane-bound organelles and other cell components. At the onset of mitosis, cytoplasmic microtubules disassemble, and the free tubulin molecules rearrange to form the mitotic spindle, which bridges between the chromosomes in the center and the centrosomes at opposite poles of the cell. The spindle remains in dynamic equilibrium with the pool of free tubulin, and therefore must constantly add tubulin subunits to function properly. During anaphase, controlled subtraction of tubulin subunits leads to contraction of the mitotic spindle and concurrent migration of the chromosomes to opposite poles of the dividing cell. However, in the presence of agents that interfere with tubulin polymerization, the depolymerization reaction quickly dominates the dynamic equilibrium process, and the mitotic spindle disappears.[6] The dividing cell is arrested in metaphase, and cell death soon follows. Even when concentrations of microtubule assembly inhibitors are too low to disrupt the morphology of the mitotic spindle, these agents can still freeze mitotic cells in metaphase. This can be explained by the crucial role microtubules play in maintaining normal cellular functions other than mitosis.[7]

There are three major classes of antimitotic agents. Microtubule-stabilizing agents, which bind to fully formed microtubules and prevent the depolymerization of tubulin subunits, are represented by paclitaxel and the taxanes, the epothilones, and eleutherobin. The other two classes function by binding to tubulin monomers and inhibiting their polymerization into microtubules. Vinca alkaloids such as vinblastine and vincristine represent one of these classes. Colchicine and colchincine-site binders define the third class of antimitotic agents. The vinca alkaloids and the colchicine-site binders occupy distinctly different sites when bound to tubulin. Both the taxanes and vinca alkaloids are widely used to treat human cancers, while colchicine-site binders are being vigorously pursued as potential new chemotherapeutic agents.[1]

During the course of a screen for agents that inhibit cell proliferation, we discovered A-105972 (**1**) (Figure 1). Competition experiments with radiolabeled colchicine confirmed that A-105972 binds to tubulin and at least partially overlaps the same binding site as colchicine.[8] Additionally, A-105972 was found to retain the same activity in multi-drug-resistant (MDR(+)) tumor cell lines as it displayed in nonresistant (MDR(−)) cell lines, which is a desirable quality for a chemotherapeutic agent. Herein, we report our work to improve the potency and in vivo efficacy shown by this new series of colchicine-site binders.

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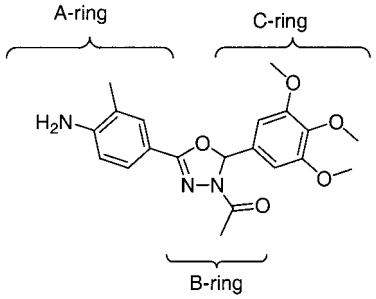


A-105972 (1)

Figure 1. Structure of A-105972 (**1**).

Chemistry

The tricyclic system was convergently assembled by first setting the substitution patterns on the flanking A- and C-rings and then closing the central B-ring. Compounds containing an oxadiazoline B-ring were prepared as follows (Scheme 1). The A-ring was derived from a benzoyl hydrazide, **2**, which could be prepared in turn by coupling a benzoic acid, **3**, with *N*-Boc-hydrazine, followed by TFA-mediated removal of the Boc group. The hydrazide **2** could be condensed with a benzaldehyde, **4**, bearing the substitution pattern desired for the C-ring. The *N*-benzoyl hydrazone **5** was then heated in acetic anhydride to effect closure of the oxadiazoline ring and complete the assembly of tricyclic system **6**. Ring closure could also be accomplished with acetic−formic anhydride to give an *N*-formyloxadiazoline, **7**, with ethyl oxalyl chloride to give an *N*-(ethyloxalamido)oxadiazoline, **8**, with chloroacetic anhydride to give an *N*-(chloroacetyl)oxadiazoline, **9**, or with dimethyl pyrocarbonate to give carbamate **10**. Other derivatives could be prepared from chloroacetate **9** by simple S_N2 displacement with a variety of nucleophiles to give compounds such as acetate **11**, azide **12**, or dimethylamine **13** (Scheme 2). *O*-Acetate hydrolysis afforded hydroxyacetate **14**. Amido acetates were prepared by reduction of the azide to amine **15** and formylation to give formamide **16** or acylation to give acetamide **17**.

The preparation of compounds containing an oxazoline B-ring started from 3,4,5-trimethoxybenzaldehyde (**18**) (Scheme 3). Henry reaction with nitromethane gave nitro alcohol **19**, which was subsequently hydrogenated to give the corresponding 2-hydroxy-2-phenethylamine **20**. *N*-Acylation with a substituted benzoic acid gave a 2-hydroxy-2-phenethylamide, **21**. Treatment of the hydroxy amide **21** with Burgess reagent or with thionyl chloride then gave the desired oxazoline-based analogues **22a**−**h.**

Other B-rings were prepared by a variety of methods. Activation of 3-methyl-4-nitrobenzoic acid (**23**) with 1,1′-carbonyldiimidazole followed by adding the lithium anion of 3,4,5-trimethoxyacetophenone gave 1,3-diketone **24** (Scheme 4). Condensation with hydrazine afforded pyrazole **25**, which could be *N*-acylated to afford a mixture of amides **26** and **27**. Hydrogenation of the nitro group and separation of the regioisomers then gave desired analogue **28**. B-rings containing a sulfur atom were prepared starting with *N*-[4-(*N*,*N*-dimethylamino)-benzoyl]-*N*′-Boc-hydrazine (**29**) (Scheme 5). Installation of the sulfur atom with Lawesson's reagent followed by Boc deprotection with trifluoroacetic acid gave thiohydrazide **30**. Condensation with 3,4,5-trimethoxybenzaldehyde (**18**) in ethanol solution presumably gave hydrazone **31**, which rapidly ring-closed and then oxidized in the presence of air to give thiadiazole **32**. When the condensation was carried out in acetic acid, the oxidation reaction was not as rapid, and addition of acetic anhydride afforded thiadiazoline **33**. Oxazoline **34** was prepared by acylation of hydrazide **35** with 3,4,5-trimethoxybenzoyl chloride, followed by dehydration of dibenzoylhydrazine **36** with $POCl_3$ to effect ring closure (Scheme 6). Dioxolane **37** was prepared starting from styrene **38**[9] (Scheme 7). Dihydroxylation of styrene **38** with OsO_4 and NMO gave diol **39**. Condensation of diol **39** with 4-(*N*,*N*-dimethylamino)benzaldehyde to close the B-ring afforded the dioxolane.

In Vitro Assays

Detailed protocols for the antiproliferation assays have been reported elsewhere.[8] Briefly, HCT-15 or NCI-H460 cells in 48- or 96-well plates containing growth medium were treated with compounds at different concentrations and then incubated for 48 h. Cells were trypsinized, and the number of cells in each well was determined using a Coulter counter. Compounds with ED_{50} values of 1 μM or less were retested in both assays.

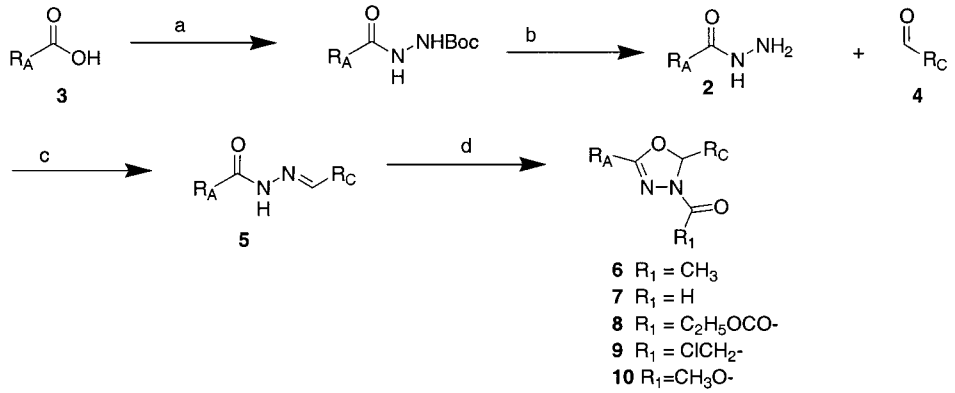
Results and Discussion

A-105972 (**1**) showed ED_{50} values of 0.029 μM against the HCT-15 (MDR(+)) cell line and 0.049 μM against the NCI-H460 (MDR(−)) cell line. In comparison, paclitaxel showed an ED_{50} of 0.015 μM against the MDR-(−) cell line, but its ED_{50} value was 0.54 μM against the MDR(+) cell line. Although the potency of compound **1** was sufficient to display efficacy in rats (see Figure 2), the lead compound had a short in vivo half-life (see Table 6). Medicinal chemistry efforts focused on modifications that could potentially improve the in vivo profile, while preserving or improving the potency of the series.

Compound **1** can be described as being composed of three subunits, the A-ring, the B-ring or oxadiazoline core, and the C-ring (Figure 1). Each region was examined to determine where changes were tolerated, and what modifications would lead to potent compounds with improved pharmacokinetic profiles. In all cases, compound purity was determined either by combustion analysis or by reversed-phase HPLC analysis.

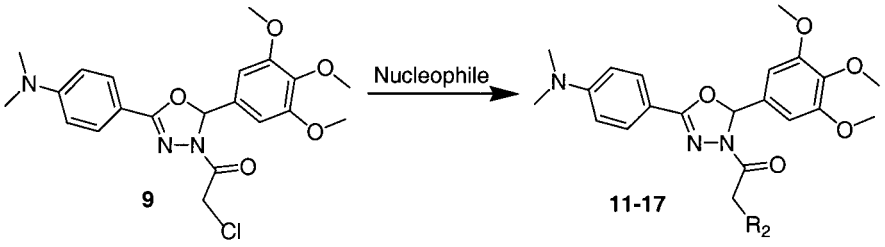
Our first A-ring analogues were prepared from the large pool of commercially available substituted benzoic acids. After several substituents which lacked the desired potency were explored, the *p*-(*N*,*N*-dimethylamino)-substituted **6a** emerged as the best of the series (Table 1). This represented a molecule that retained all of the potency of compound **1**, but was much easier to prepare. A positional survey showed that the optimal placement for the dimethylamino group was in the para position. When the dimethylamino group was moved to the ortho position as in **6b**, the result was a 1000-fold loss of potency. The corresponding meta-substituted compound **6c** was slightly less potent than *p*-(*N*,*N*-dimethylamino) **6a**. More detailed studies on the mechanism of action for compound **1** and compound **6a**, including cell cycle analysis, assays of microtubule assembly, electron microscopy, and apoptosis studies, have been reported elsewhere.[8,10] These studies clearly

Scheme 1[a]



[a] Reagents and conditions: (a) H_2NNH_2Boc, EDCI, DMF; (b) TFA, CH_2Cl_2; (c) acetic acid, EtOH; (d) Ac_2O (**6**), Ac_2OHCO_2H (**7**), ethyl oxalyl chloride (**8**), chloroacetic anhydride (**9**), or dimethyl pyrocarbonate (**10**).

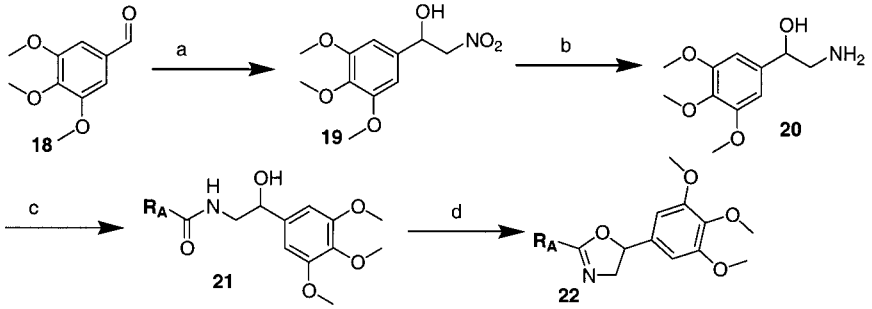
Scheme 2[a]



Nucleophile	Solvent	Compound	R_2
KOAc	DMF	**11**	OAc
NaN_3	DMF	**12**	N_3
$(CH_3)_2NH$	THF	**13**	$(CH_3)_2N$
		14	OH[a]
		15	NH_2[b]
		16	NHCHO[c]
		17	$NHCOCH_3$[d]

[a] Reagents and conditions: (a) by hydrolysis of acetate **11** with K_2CO_3 in aqueous MeOH; (b) by reduction of azide **12** with PPh_3 in THF; (c) by formylation of amine **15** with formic acid and EDAC in CH_3CN; (d) by acylation of amine **15** with acetic anhydride.

Scheme 3[a]

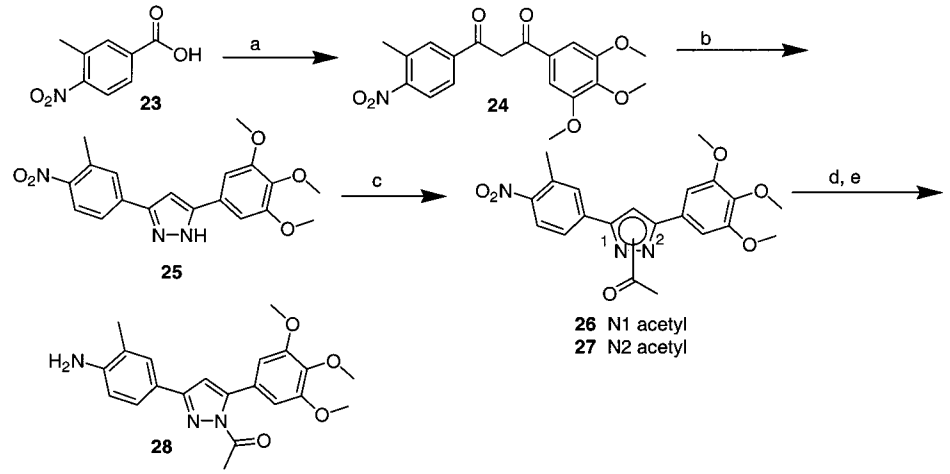


[a] Reagents and conditions: (a) (i) CH_3NO_2, 10% aqueous NaOH, EtOH; (ii) AcOH (70%); (b) 60 psi of H_2/Pd−C, AcOH (94%); (c) R_ACOCl, $(i\text{-Pr})_2EtN$, CH_2Cl_2 or R_ACOOH, EDAC, $i\text{-PrEt}_2N$, CH_2Cl_2; (d) Burgess reagent, $CHCl_3$ or $SOCl_2$, $CHCl_3$.

demonstrated that both compounds inhibit microtubule assembly and freeze the cell cycle in metaphase before inducing apoptosis.

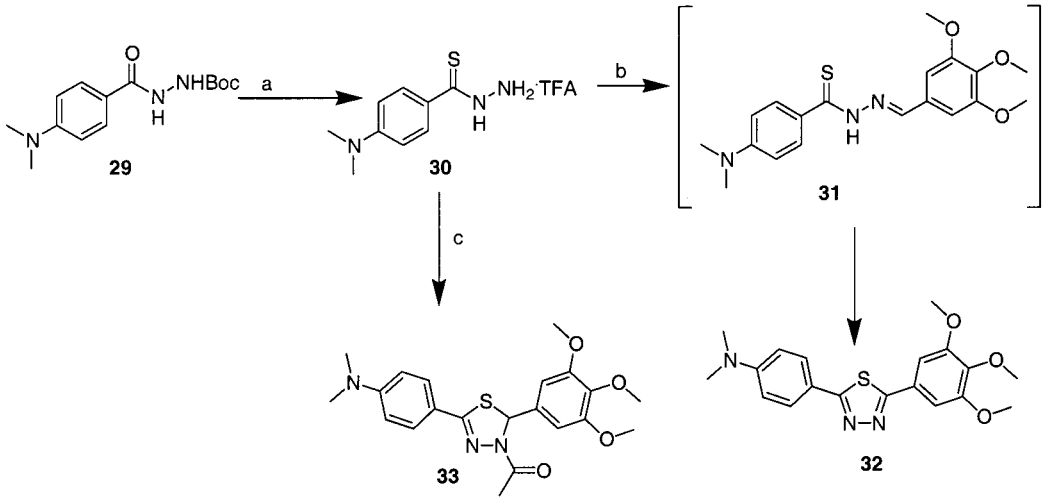
Structure−activity relationship (SAR) studies on the C-ring demonstrated that the 3,4,5-trimethoxy substitution pattern was apparently optimal (Table 2). For example, 2,4,5-trimethoxyphenyl **6d**, 2,4,6-trimethoxyphenyl **6e**, and 2,3,4-trimethoxyphenyl **6f** were at least 100-fold less potent than 3,4,5-trimethoxyphenyl **1**. Little change was tolerated when we replaced or removed any of the methoxy groups. *p*-Amide **6g**, 3,4,5-trimethyl **6h**, acetoxy compound **6i**, and 3,4-dimethoxyphenyl **6j** all showed very weak activity, but 4-carboxylic ester **6k** retained some potency. Even methylenedioxy substitution for two of the methoxy groups in **6l** produced an unacceptable loss of potency. This SAR about the C-ring was reminiscent of that observed with colchicine. The trimethoxyphenyl ring is crucial for retaining potency in this and in other series of molecules which occupy the colchicine binding site.[11]

Scheme 4[a]



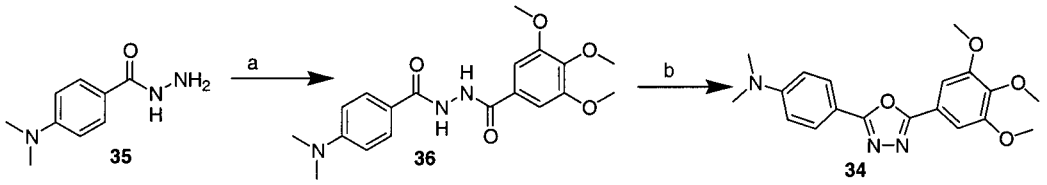
[a] Reagents and conditions: (a) (i) CDI, THF; (ii) LDA, 3,4,5-trimethoxyacetophenone, THF; (b) $H_2NNH_2 \cdot H_2O$, CH_2Cl_2/EtOH; (c) Ac_2O; (d) H_2/Pd−C, EtOAc (100%); (e) separate regioisomers by flash chromatography.

Scheme 5[a]



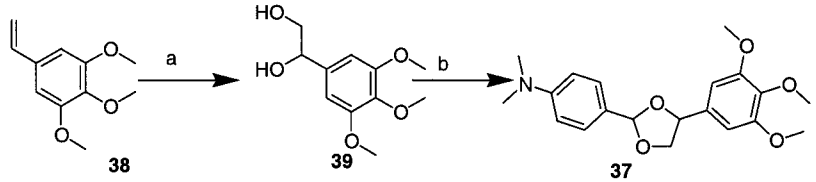
[a] Reagents and conditions: (a) (i) Lawesson's reagent; (ii) TFA, CH_2Cl_2; (b) **18**, EtOH; (c) (i) **18**, AcOH, under argon; (ii) Ac_2O, 95 °C.

Scheme 6[a]



[a] Reagents and conditions: (a) 3,4,5-trimethoxybenzoyl chloride; (b) $POCl_3$.

Scheme 7[a]



[a] Reagents and conditions: (a) OsO_4, NMO; (b) 4-(*N*,*N*-dimethylamino)benzaldehyde, TsOH, toluene, 4 Å molecular sieves.

Replacement of the *N*-acetyl group present on the B-ring in compound **6a** gave several compounds that retained activity (Table 3). *N*-Formyl **7**, *N*-(hydroxyacetyl) **14**, *N*-(formamidoacetyl) **16**, and *N*-(acetoxyacetyl) **11** all showed approximately the same potency as compound **1**. *N*-(azidoacetyl) **12**, *N*-(acetamidoacetyl) **17**, and *N*-(aminoacetyl) **15** were about 5−10-fold less active than compound **6a**, but *N*-[(*N*,*N*-dimethylamino)-acetyl] **13**, *N*-(methoxycarbonyl) **10**, and *N*-ethyloxalamide **8** lost all activity. On this portion of the molecule,

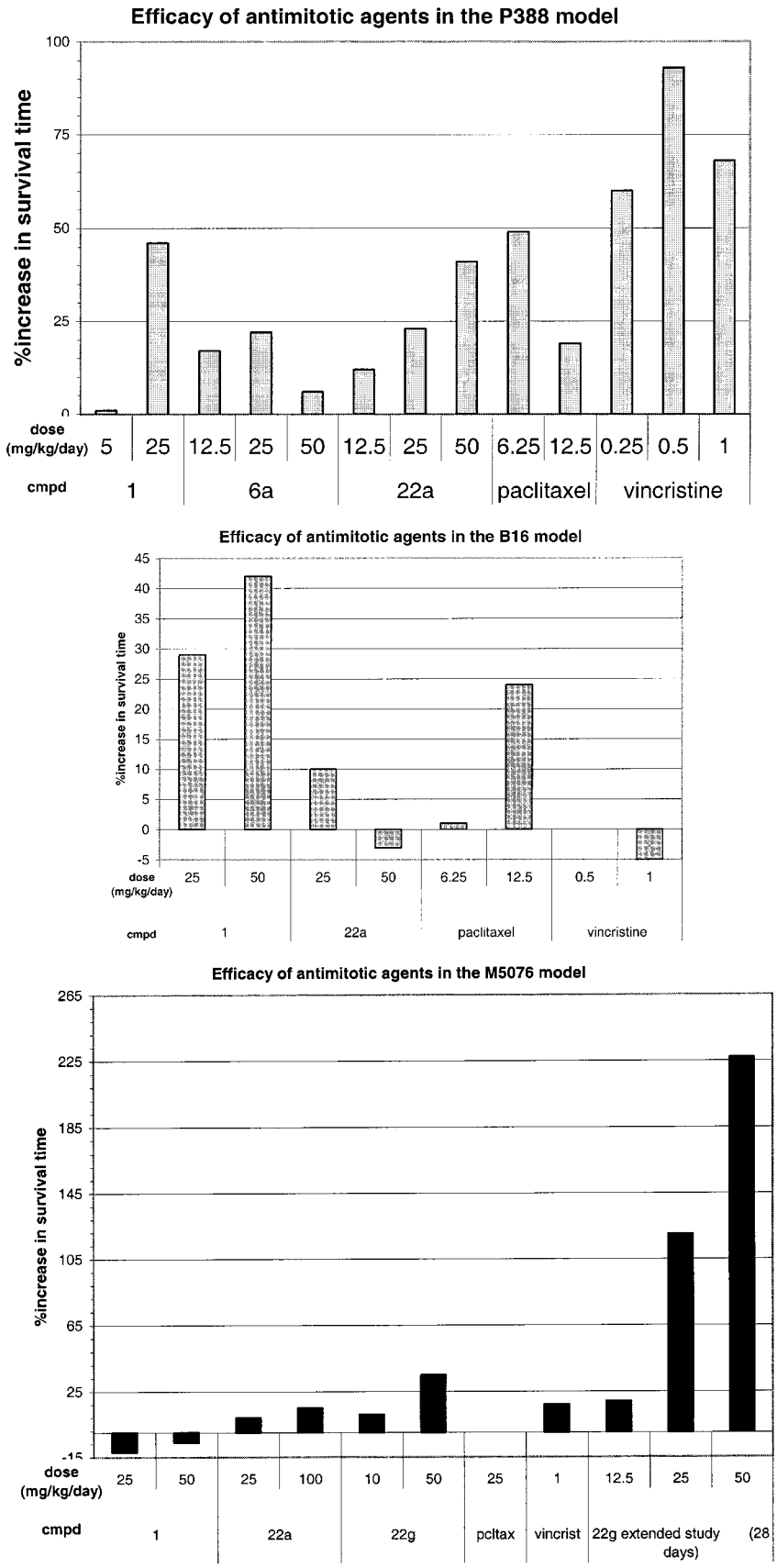
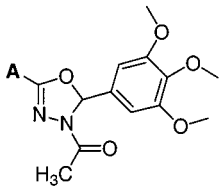
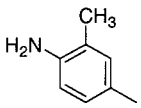
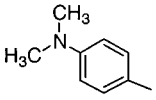
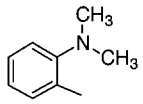
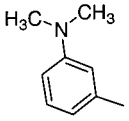


Figure 2. In vivo efficacy of antimitotic agents in various murine models. Each bar represents a treatment group of 10 mice. In each model, a lifespan increase of 25% or greater was considered biologically significant. For the P388 model, the tumor was intraperitoneal, and compounds were dosed ip, qd for 5 days (**22a**, vincristine, paclitaxel), 7 days (compound **1**), or 9 days (compound **6a**). For the B16 model, compound **1** was dosed against an intraperitoneal tumor, while other compounds were dosed against a subcutaneous tumor. Also in the B16 model, compounds were dosed ip, qd for 5 days. For the M5076 model, paclitaxel and vincristine were dosed against an intraperitoneal tumor ip, qd. Compounds **1**, **22a**, and **22g** were dosed against a subcutaneous tumor according to the following regimens: compound **1** was dosed ip, qd; compounds **22a** and **22g** were dosed po, qd.

Table 1. SAR of A-Ring Substitutions



		ED_{50} (μM)±SD	
Compound	A-ring	HCT-15	NCI-H460
Paclitaxel		0.54±0.48	0.015±0.006
1		0.042±0.02	0.059±0.02
6a		0.033±0.12	0.044±0.02
6b		65	109
6c		0.16±0.098	0.26±0.17

small, uncharged groups were acceptable additions to the *N*-acetate group, but such changes did not improve potency relative to the lead compound.

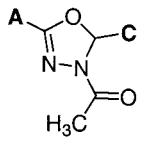
The potential for the oxadiazoline ring to undergo hydrolysis in vivo was seen as a major liability with the lead molecule, so the search for a replacement for the B-ring was a major goal of our SAR study. Some of the analogues we prepared bore the A-ring found in compound **1**, while others contained the 4-(*N*,*N*-dimethylamino)phenyl A-ring found in **6a**. Either of these A-rings was active when attached to the oxadiazoline B-ring, but analogues with a 4-(*N*,*N*-dimethylamino)-phenyl A-ring were generally easier to prepare. Table 4 gives several examples of heterocyclic replacements for the oxadiazoline ring. Aromatic heterocycles such as oxadiazole **34**, thiadiazole **32**, and pyrazole **28** were inactive. Thiadiazoline **33** showed modest activity, but 1,3-oxazoline **22a** retained most of the activity seen with the original oxadiazoline B-ring. In contrast, dioxolane **37** was much less potent than oxazoline **22a**. These results demonstrate that both the 1-oxygen and 3-nitrogen atoms are required for activity, but the 4-nitrogen atom and the pendant acetate are not required. Additionally, the B-ring must have an sp^3 carbon at the 5-position, rendering aromatic analogues inactive.

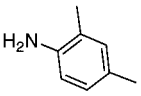
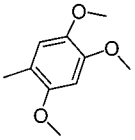
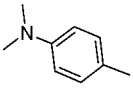
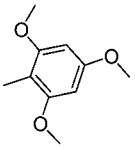
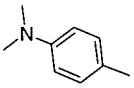
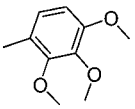
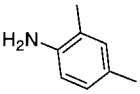
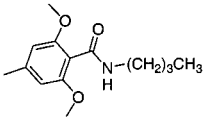
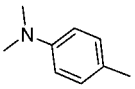
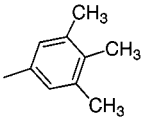
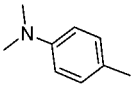
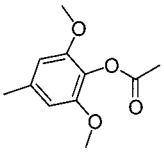
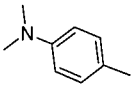
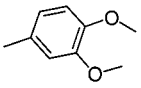
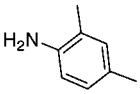
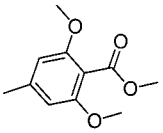
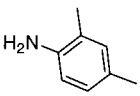
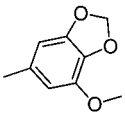
Following the discovery of oxazoline **22a**, we continued to explore the SAR of the A-ring coupled to the oxazoline B-ring (Table 5). We explored placing an unsubstituted amino group in the A-ring 3-position. Both 3-amino-4-methoxy **22b** and 3-amino-4-methyl **22c** were active compounds, but they proved to be more prone to decomposition than the 4-amino analogues. We tried to make additional gains by taking advantage of alkyl substitutions on the 4-amino group. It was easy to prepare a large number of compounds via palladium-mediated aminations on the corresponding 4-bromide,[12] but all of these analogues lost a great deal of activity in the antimitotic assay. Reasoning that the coplanar orientation of the *N*,*N*-dimethyl groups with the benzene ring might be critical, we investigated fusing nitrogen-containing heterocycles to the A-ring. Benzoxazolone **22d**, tetrahydroquinoline **22e**, and indoline **22f** were all less active than **22a**, but indoles **22g** and **22h** were each as potent as compound **22a**. Clearly, both *N*-alkyl substituents were not necessary for activity, but only the rigid, electron-rich indole maintained activity out of all the A-ring heterocycles we examined.

In Vivo Results. On the basis of the trends we saw in vitro, we chose to examine the in vivo profiles of compounds **1**, **6a**, **22a**, **16**, **14**, and **22g**. We began with the determination of the pharmacokinetic parameters for the new compounds. Unfortunately, the physical properties of substituted acetates **16** and **14** made it difficult to dose these agents safely. Both of these compounds were insoluble either in an ethanol/propylene glycol/D5W medium or a DMSO/PEG400/D5W medium, and attempted dosing of a suspension led to mortality in rats. Compounds **1**, **6a**, **22a**, and **22g** all displayed pharmacokinetic profiles that allowed us to proceed with evaluation of their efficacies in murine tumor models (Table 6).The new antimitotic agents, paclitaxel, and vincristine were evaluated in P388, B16, or M5076 murine cancer models. Approximately 8 week old mice were inoculated with tumor cells on day 0, and then therapy was initiated on day 1 of the study. Mice were monitored daily for mortality, and were euthanized when moribund. The day of death was recorded for each individual, and was used to calculate the percent increase in life span (ILS). A 25% or greater increase in life span was considered biologically significant.

Early studies were conducted using the P388 and B16 tumor models (Figure 2). Both compound **1** and compound **22a** showed dose-dependent in vivo activity in the P388 model, but compound **6a** showed no significant activity. Paclitaxel and vincristine were both active in the P388 model. In the B16 model, again compound **1** showed dose-dependent activity, but compound **22a** failed to show any activity. Paclitaxel retained its anticancer activity in the B16 model, but vincristine was not active. M5076 is a faster growing tumor model that presents a higher standard of efficacy for in vivo agents than either P388 or B16 tumor models. In the M5076 model, compound **1**, compound **22a**, paclitaxel, and vincristine were all inactive. However, compound **22g** showed efficacy at 50 (mg/kg)/day (Figure 2). Significantly, this activity was apparent following oral dosing of compound **22g**. Additionally, the compound was efficacious in a solid tumor model. Inspired by this result, we evaluated compound **22g** in an extended M5076 study. After a 28 day dosing regimen was complete, the dose-dependent efficacy of compound **22g** became clear, with an increase in mean survival time of 120% at 25 (mg/kg)/day and 227% at 50 (mg/kg)/day.

Table 2. SAR of C-Ring Substitutions

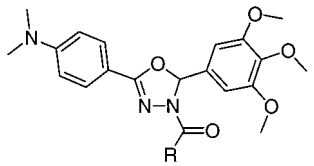


			ED_{50} (μM)±SD	
Compound	A-ring	C-ring	HCT-15	NCI-H460
6d			198	1000
6e			71	40
6f			7.5	6.8
6g			178	60
6h			1.5	5.7
6i			78	13
6j			8.2	5.1
6k			0.28±0.14	0.51±0.25
6l			0.62±0.13	0.63±0.23

Conclusions

A new series of tubulin polymerization inhibitors with activity in multi-drug-resistant tumor cell lines has been discovered. Medicinal chemistry efforts starting from lead compound **1** led to the development of several compounds worthy of in vivo evaluation. Structural modifications gave us compounds with slightly better in vitro activity against both MDR(−) and MDR(+) cell lines, but in vivo efficacy was improved greatly over those of the lead compound, paclitaxel, and vincristine. In M5076 cancer models, indolyloxazoline **22g** (A-259745) rose above the other compounds we evaluated

Table 3. SAR of *N*-Acetate Substitutions



		ED_{50} (μM) ± SD	
compd	R	HCT-15	NCI-H460
7	H	0.096 ± 0.06	0.12 ± 0.09
8	**COOC**$_2$H$_5$	45	33
10	OCH$_3$	40	106
11	**CH**$_2$OAc	0.019 ± 0.004	0.072 ± 0.005
12	**CH**$_2$N$_3$	0.15 ± 0.02	0.53 ± 0.03
13	**CH**$_2$N(CH$_3$)$_2$	80	74
14	**CH**$_2$OH	0.026 ± 0.016	0.043 ± 0.028
15	**CH**$_2$NH$_2$	0.65 ± 0.31	0.66 ± 0.068
16	**CH**$_2$NHCHO	0.035 ± 0.028	0.040 ± 0.03
17	**CH**$_2$NHAc	0.18 ± 0.04	0.48 ± 0.01

and increased the life span of mice up to a factor of 3 during a 28 day study. Compound **22g** represents a new tubulin polymerization inhibitor with oral in vivo activity against solid tumors and a straightforward chemical synthesis.[13] This agent, and others of its structural class, may prove useful in the development of new chemotherapeutic drugs.

Experimental Section

Analytical HPLC traces were obtained by elution through a C_{18} reversed-phase column with a gradient of 5 mM aqueous ammonium acetate/acetonitrile or 0.1% aqueous TFA/acetonitrile as eluant. The compounds were purified to homogeneity unless otherwise indicated. ^{1}H NMR and MS data were recorded at Abbott Laboratories. Combustion analysis data were obtained from Robertson Microlit Laboratories, Inc., Madison, NJ.

2-(4-Amino-3-methylphenyl)-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (1).[14] A solution of 411 mg (1 mmol) of 2-(4-azido-3-methylphenyl)-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (see the general experimental procedures for hydrazides **2**, hydrazones **5**, and oxadiazolines **6**) in 5 mL of acetonitrile and 5 mL of THF was prepared. This was added to a stirred suspension of 283 mg (1.5 mmol) of $SnCl_2$, 616 μL (6 mmol) of thiophenol, and 627 μL (4.5 mmol) of triethylamine in 5 mL of acetonitrile. After being stirred for 1 h, the reaction was quenched by addition of 10 mL of 1 M NaOH. The solution was poured into additional 1 M NaOH and then extracted with EtOAc. The organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The product was purified by silica gel chromatography, eluting with a 55−70% gradient of ethyl acetate/hexanes, to give 310 mg (80%) of compound **1**. ^{1}H NMR and MS data were identical to those of a commercially obtained sample of compound **1**: ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.07 (s, 3H), 2.24 (s, 3H), 3.66 (s, 3H), 3.75 (s, 6H), 5.64 (s, 2H), 6.64 (d, 1H, J = 7.8 Hz), 6.72 (s, 2H), 6.98 (s, 1H), 7.36−7.39 (m, 2H); MS (ESI) m/z 386 [M + H]$^+$.

4-Azido-3-methylbenzoic Acid. To a suspension of 1 g (6.6 mmol) of 4-amino-3-methylbenzoic acid in 1 mL of diethyl ether was added ethereal diazomethane until effervescence ceased. The solvent was removed in vacuo, giving 1.05 g (96%) of methyl 4-amino-3-methylbenzoate as a crystalline solid.

To an ice-cooled suspension of 1.05 g (6.4 mmol) of methyl 4-amino-3-methylbenzoate in 15 mL of 6 N sulfuric acid was added a solution of 485 mg (7.0 mmol) of $NaNO_2$ in 1.4 mL of water, keeping the temperature below 5 °C. The solution was stirred for 45 min, and then a solution of 529 mg (8.1 mmol) of NaN_3 in 1.7 mL of water was added, keeping the temperature below 5 °C, and keeping the vigorous effervescence under control. The product suspension was stirred for 10 min and then extracted with diethyl ether. The ether layers were concentrated to 1.09 g (90%) of a tan crystalline solid.

To a solution of 1.09 g (5.31 mmol) of methyl 4-azido-3-methylbenzoate in 6 mL of ethanol was added 2 mL (19.8 mmol) of 25% aqueous NaOH. The reaction was stirred at ambient temperature for 1 h, and then the solvents were removed in vacuo. The residue was taken up in water and washed with diethyl ether, and then the aqueous layer was acidified to pH 1 by addition of 1 N H_2SO_4. The azido acid was extracted into diethyl ether, and the second set of ether layers was dried over Na_2SO_4, filtered, and concentrated to 965 mg (95%) of 4-azido-3-methylbenzoic acid as a tan crystalline solid.

3-Methyl-4-azidobenzoyl Hydrazide (General Procedure for the Preparation of Hydrazides 2). To a solution of 0.965 g (5.45 mmol) of 4-azido-3-methylbenzoic acid in 5 mL of DMF and 5 mL of THF was added 0.755 g (5.72 mmol) of *tert*-butylcarbazate, 1.15 g (5.99 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), and 10 mg (0.08 mmol) of 4-(*N*,*N*-dimethylamino)pyridine. The reaction was stirred at ambient temperature for 17 h and then poured into a mixture of 50 mL of ice cold water and 50 mL of diethyl ether. The layers were separated, and then the ether layer was washed with 1 M aqueous $NaHSO_4$, water, saturated aqueous $NaHCO_3$ solution, and brine. The ether layer was dried over Na_2SO_4, filtered, and concentrated to a white solid.

The white solid was suspended in 20 mL of CH_2Cl_2, and cooled with an ice bath. Next, 15 mL of trifluoroacetic acid was added, and the mixture was stirred for 2 h at 0 °C. The solvents were removed in vacuo, and then the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$ solution. The organic phase was washed with water and then brine, dried over Na_2SO_4, filtered, and concentrated to give 1.02 g (98%) of 4-azido-3-methylbenzoyl hydrazide as a tan crystalline solid.

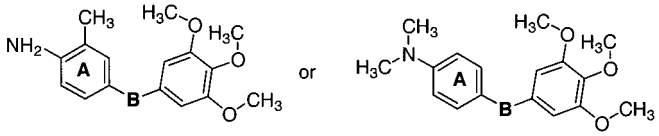
4-(Azidomethyl)-3-methylbenzoic Acid (3,4,5-Trimethoxybenzylidene)hydrazide (General Procedure for the Preparation of Hydrazones 5). To a solution of 1.02 g (5.34 mmol) of 3-methyl-4-azidobenzoyl hydrazide in 50 mL of ethanol and 2 mL of acetic acid was added 1.05 g (5.34 mmol) of 3,4,5-trimethoxybenzaldehyde. The solids dissolved, and then a white precipitate quickly formed. After being stirred for 20 min, the mixture was cooled to 10 °C. The precipitate was collected, washed with cold ethanol, and dried in vacuo to give 1.35 g (67%) of the hydrazone: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.25 (s, 3H), 3.90 (s, 9H), 6.97 (br s, 2H), 7.18 (d, 1H, J = 7 Hz), 7.73 (br s, 3H), 9.16 (br s, 1H); MS (DCI/NH_3) m/z 369 [M + H]$^+$.

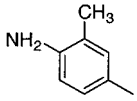
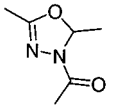
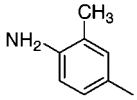
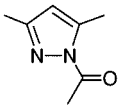
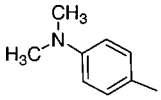
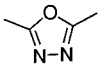
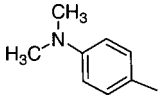
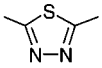
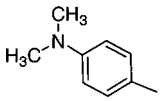
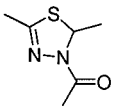
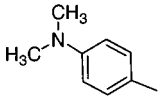
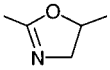
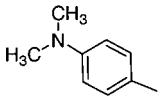
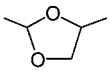
2-(4-Azido-3-methylphenyl)-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (General Procedure for the Preparation of Oxadiazolines 6). To 850 mg (2.30 mmol) of 4-(azidomethyl)-3-methylbenzoic acid (3,4,5-trimethoxybenzylidene)hydrazide was added 15 mL of acetic anhydride. The reaction was heated to 125 °C, and the starting hydrazide dissolved. After the solution was stirred at 125 °C for 2 h, the solvent was removed in vacuo. The residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$, and then the layers were separated. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified via silica gel chromatography, eluting with 45% ethyl acetate/hexanes, to give 605 mg (64%) of 2-(4-amino-3-methylphenyl)-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (6a). 6a was prepared according to the general procedures for compounds **2**, **5**, and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.37 (s, 3H), 3.03 (s, 6H), 3.82 (s, 3H), 3.85 (s, 6H), 6.68 (d, 2H, J = 7 Hz), 6.72 (s, 2H), 6.96 (s, 1H), 7.65 (d, 2H, J = 7 Hz); MS (CDI/NH_3) m/z 400 [M + H]$^+$. Anal. Calcd for $C_{21}H_{25}N_3O_5$: C, 63.13; H, 6.31; N, 10.52. Found: C, 63.05; H, 5.95; N, 10.31.

2-[2-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (6b). 6b was prepared according to the general procedures for compounds **2**, **5**, and

Table 4. SAR of B-Ring Substitutions



			ED_{50} (μM)±SD	
Compound	A-ring	B-ring	HCT-15	NCI-H460
1			0.04±0.02	0.059±0.02
28			365	1000
34			92	106
32			231	1000
33			0.61±0.20	0.35±0.17
22a			0.12±0.14	0.35±0.17
37			2.4	8.3

6, starting with 2-(*N*,*N*-dimethylamino)benzoic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.40 (s, 3H), 2.84 (s, 6H), 3.82 (s,3H), 3.84 (s, 6H), 6.72 (s, 2H), 6.86 (s, 1H), 7.06 (d, 1H, J = 9 Hz), 7.42 (t, 1H, J = 9 Hz), 7.74 (d, 1H, J = 9 Hz). MS (DCI/NH_3) m/z 400 $[M + H]^+$.

2-[3-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (6c). 6c was prepared according to the general procedures for compounds **2**, **5**, and **6,** starting with 3-(*N*,*N*-dimethylamino)benzoic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 3.01 (s, 6H), 3.83 (s, 3H), 3.87 (s, 6H), 6.72 (s, 2H), 6.85 (d, 1H, J = 7 Hz), 6.99 (s,1H), 7.19 (br s, 1H), 7.26−7.33 (m, 2H). MS (CDI/NH_3) m/z 400 $[M + H]^+$.

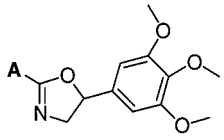
2-(3-Methyl-4-aminophenyl)-4-acetyl-5-(2,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (6d). 6d was prepared according to the general procedures for compounds **2**, **5**, and **6**, starting with 3-methyl-4-azidobenzoyl hydrazide and 2,4,5-trimethoxybenzaldehyde. The product obtained from the oxadiazoline synthesis was hydrogenated using 1 atm of H_2/Pd−C in ethyl acetate to give the desired product: ^{1}H NMR (d_6-DMSO, 300 MHz) δ 2.03 (s, 3H), 2.22 (s, 3H), 3.63 (s, 3H), 3.78 (s, 3H), 3.83 (s, 3H), 5.60 (s, 2H), 6.62 (d, 1H, J = 8 Hz), 6.72 (s, 1H), 6.76 (s, 1H), 7.08 (s, 1H), 7.32 (d, 1H, J = 8 Hz), 7.37 (s, 1H); MS (DCI/NH_3) m/z 386 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(2,4,6-trimethoxyphenyl)-Δ2,3-oxadiazoline (6e). 6e was prepared according to the general procedures for compounds **5** and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoyl hydrazide and 2,4,6-trimethoxybenzaldehyde: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (m, 2H), 7.52 (s, 1H), 6.67 (m, 2H), 6.11 (s, 2H), 3.79 (s, 3H), 3.76 (s, 6H), 3.02 (s, 6H), 2.25 (s, 3H); MS (DCI/NH_3) m/z 400 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(2,3,4-trimethoxyphenyl)-Δ2,3-oxadiazoline (6f). 6f was prepared according to the general procedures for compounds **5** and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoyl hydrazide and 2,3,4-trimethoxybenzaldehyde: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (m, 2H), 7.16 (s, 1H), 7.02 (d, 1H, J = 8.4 Hz), 6.67−6.64 (m, 3H), 3.94 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.02 (s, 6H), 2.35 (s, 3H); MS (DCI/NH_3) m/z 400 $[M + H]^+$.

3,5-Dimethoxy-4-[[*N*-(*n*-butyl)amino]carbonyl]benzaldehyde. To a solution of 2.12 g (10 mmol) of 4-(hydroxymethyl)-2,6-dimethoxybenzoic acid in 22 mL of DMF was added 4.05 g (30 mmol) of benzotriazol-1-ol, 1.01 g (10 mmol) of *N*-methylmorpholine, and 1.91 g (10 mmol) of EDCI. The reaction was stirred at ambient temperature for 18 h, then diluted with saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The combined ethyl acetate layers were back-

Table 5. SAR of A-Ring Substitutions with an Oxazoline B-Ring



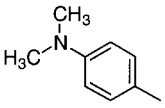
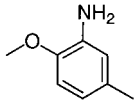
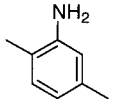
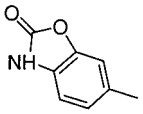
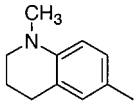
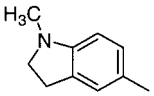
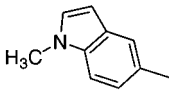
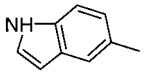
		ED_{50} (μM)±SD	
Compound	A-ring	HCT-15	NCI-H460
22a		0.12±0.14	0.35±0.17
22b		0.082±0.023	0.073±0.019
22c		0.082±0.053	0.063±0.017
22d		4.3	72
22e		0.66±0.02	0.32±0.15
22f		0.61±0.17	0.82±0.26
22g		0.018±0.017	0.028±0.018
22h		0.097±0.063	0.116±0.07

Table 6. Pharmacokinetic Parameters for Compounds Tested in Vivo

			intravenous			oral	
compd	$t_{1/2}$[a] (h)	V_c[b] (L/kg)	AUC[c] [(ng h)/mL]	C_{max}[d] (ng/mL)	T_{max}[e] (h)	AUC[f] [(ng h)/mL]	F[g] (%)
1	1.1	2.2	2109	59	1.7	147	7
6a	**1.1**	3.3	3170	113	0.4	155	4.9
22a	0.8	3.3	3407	891	0.9	1657	49
22g	1.9	1.4	5119	799	0.4	1777	35

[a] Half-life following intravenous dosing. [b] Volume of distribution following intravenous dosing. [c] Area under the curve following intravenous dosing, integrated drug concentration with respect to time. [d] Maximum plasma concentration following intravenous dosing. [e] Time to maximum plasma concentration following oral dosing. [f] Integrated drug concentration with respect to time following oral dosing. [g] Percent oral bioavailability.

extracted with water and then brine, dried over Na_2SO_4, filtered, and concentrated to give 1.70 g (64%) of *N*-butyl-4-(hydroxymethyl)-2,6-dimethoxybenzamide as a white solid.

To a suspension of 1.70 g (6.4 mmol) of *N*-butyl-4-(hydroxymethyl)-2,6-dimethoxybenzamide in 50 mL of CH_2Cl_2 and 10 mL of $CHCl_3$ was added 2.30 g (10.7 mmol) of pyridinium chlorochromate. The mixture was stirred at ambient temperature for 2 h, then diluted with 50 mL of diethyl ether, and filtered to remove the dark solids. The solids were washed with ethyl acetate, and then the combined organic layers were concentrated in vacuo to give 1.5 g (89%) of 3,5-dimethoxy-4-[[*N*-(*n*-butyl)amino]carbonyl]benzaldehyde as a brown solid. This aldehyde was used without further purification for the preparation of oxadiazoline **6g**.

2-(3-Methyl-4-aminophenyl)-4-acetyl-5-[3,5-dimethoxy-4-[[*N*-(*n*-butyl)amino]carbonyl]phenyl]-Δ2,3-oxadiazoline (6g). 6g was prepared according to the general procedures for compounds **5** and **6**, substituting 3,5-dimethoxy-4-[[*N*-(*n*-butyl)amino]carbonyl]benzaldehyde for 3,4,5-trimethoxybenzaldehyde. The azido product obtained from the oxadiazoline synthesis was hydrogenated using 1 atm of H_2/Pd−C in ethyl acetate to give the desired product: ^{1}H NMR (d_6-DMSO, 300 MHz) δ 0.75 (t, 3H, J = 7.5 Hz), 1.08−1.17 (m, 2H), 1.32−1.42 (m, 2H), 2.12 (s, 3H), 2.28 (s, 3H), 3.84 (s, 6H), 5.58 (s, 2H), 6.63 (d, 1H, J = 7.5 Hz), 7.03 (s, 2H), 7.55 (m, 2H), 8.38 (s, 1H); MS (ESI) m/z 453 $[M - H]^-$; HRMS calcd for $C_{24}H_{31}O_5N_4$ + H 455.2294, found 455.2285.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4,5-trimethylphenyl)-Δ2,3-oxadiazoline (6h). 6h was prepared according to the general procedures for compounds **5** and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoyl hydrazide and 3,4,5-trimethylbenzaldehyde: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (m, 2H), 7.12 (s, 1H), 7.03 (s, 1H), 6.97 (s, 1H), 6.66 (m, 2H), 3.02 (s, 6H), 2.45 (s, 3H), 2.36 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H); MS (DCI/NH_3) m/z 352 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(4-acetoxy-3,5-dimethoxyphenyl)-Δ2,3-oxadiazoline (6i). 6i was prepared according to the general procedures for compounds **5** and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoyl hydrazide and 3,5-dimethoxy-4-hydroxybenzaldehyde: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24 (m, 2H), 6.99 (s, 1H), 6.75 (s, 2H), 6.69 (m, 2H), 3.80 (s, 6H), 3.04 (s, 6H), 2.35 (s, 3H), 2.32 (s, 3H); MS (DCI/NH_3) m/z 428 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4-dimethoxyphenyl)-Δ2,3-oxadiazoline (6j). 6j was prepared according to the general procedures for compounds **5** and **6**, starting with 4-(*N*,*N*-dimethylamino)benzoyl hydrazide and 3,4-dimethoxybenzaldehyde: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (m, 2H), 7.04 (dd, 1H, J = 2.2, 8.3 Hz), 7.00 (d, 1H, J = 2.2 Hz), 6.98 (s, 1H), 6.86 (d, 1H, J = 8.4 Hz), 6.68 (m, 2H), 3.86 (s, 6H), 3.04 (s, 6H), 2.34 (s, 6H); MS (DCI/NH_3) m/z 370 $[M + H]^+$.

2-(3-Methyl-4-aminophenyl)-4-acetyl-5-[4-(methoxycarbonyl)-3,5-dimethoxyphenyl]-Δ2,3-oxadiazoline (6k). 6k was prepared according to the general procedures for compounds **5** and **6**, substituting 3,5-dimethoxy-4-(methoxycarbonyl)benzaldehyde for 2,4,5-trimethoxybenzaldehyde. The azido product obtained from the oxadiazoline synthesis was hydrogenated using 1 atm of H_2/Pd−C in ethyl acetate to give the desired product: ^{1}H NMR (d_6-DMSO, 300 MHz) δ 2.06 (s, 3H), 2.24 (s, 3H), 3.76 (s, 9H), 5.63 (s, 2H), 6.63 (d, 1H, J = 8 Hz), 6.76 (s, 2H), 7.03 (s, 1H), 7.38 (d, 1H, J = 8 Hz), 7.40 (s, 1H); MS (DCI/NH_3) m/z 439 $[M + H]^+$.

2-(4-Amino-3-methylphenyl)-4-acetyl-5-(7-methoxybenzo[1,3]dioxol-5-yl)-Δ2,3-oxadiazoline (6l). 6l was prepared according to the general procedures for compounds **5** and **6**, substituting 3,4-(methylenedioxy)-5-methoxybenzaldehyde for 2,4,5-trimethoxybenzaldehyde. The azido product obtained from the oxadiazoline synthesis was hydrogenated using 1 atm of H_2/Pd−C in ethyl acetate to give the desired product: ^{1}H NMR (d_6-DMSO, 300 MHz) δ 2.04 (s, 3H), 2.22 (s, 3H), 3.84 (s, 3H), 5.66 (s, 2H), 6.02 (s, 2H), 6.58−6.64 (m, 2H), 6.77 (s, 1H), 6.96 (s, 1H), 7.38 (m, 2H); MS (DCI/NH_3) m/z 370 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-formyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (7). This compound was prepared by the same general procedure used to prepare

N-acetyloxadiazolines **6**, substituting a 1:1 mixture of formic acid and acetic anhydride for acetic anhydride, and then heating at reflux. The product was obtained as white crystals from hot EtOAc: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.07 (s, 6H), 3.84 (s, 3H), 3.86 (s, 6H), 6.68 (d, 2H, J = 12 Hz), 6.72 (s, 1H), 6.94 (s, 1H), 7.73 (d, 2H, J = 12 Hz), 8.77 (s, 6H); MS (DCI/NH_3) m/z 386 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-[(ethylcarboxy)-carbonyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (8). To a suspension of 651 mg of the 4-(*N*,*N*-dimethylamino)benzoyl hydrazone derived from 3,4,5-trimethoxybenzaldehyde (prepared using the general procedure for hydrazones **5**) in 8 mL of DMF was added 500 μL of ethyl oxalyl chloride. The reaction was stirred until dissolution was complete, and then stirring was continued for another 10 min. The solution was diluted with 80 mL of water and then extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate layers were back-extracted with water (1 × 20 mL), saturated $NaHCO_3$ solution (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated to a yellow oil. This was purified via silica gel chromatography, eluting with 50% ethyl acetate/hexanes, to give 433 mg of a crystalline solid. The product could also be crystallized from methanol: ^{1}H NMR ($CDCl_3$) δ (major rotamer) 1.42 (t, 3H, J = 7.0 Hz), 3.05 (s, 6H), 3.85 (s, 3H), 3.86 (s, 6H), 4.41 (m, 2H), 6.67 (m, 2H), 6.73 (s, 2H), 6.96 (s, 1H), 7.73 (m, 2H); MS (DCI/NH_3) m/z 458 $[M + H]^+$. Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.39; H, 5.95; N, 9.18. Found: C, 60.30; H, 5.97; N, 9.19.

4-(Chloroacetyl)-2-[4-(*N*,*N*-dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (9). To 1.5 g (4.2 mmol) of the 4-(*N*,*N*-dimethylamino)benzoyl hydrazone derived from 3,4,5-trimethoxybenzaldehyde (prepared according to the general procedure used for hydrazones **5**) was added 4.5 g (26.3 mmol) of chloroacetic anhydride. The mixture was stirred at 95 °C for 20 min and then poured into 50 mL of saturated aqueous $NaHCO_3$ solution, along with 10 mL of ethyl acetate. After the resulting mixture was stirred for 1 h, the precipitate was filtered and washed with ethyl acetate and then with ethanol to give 1.01 g (55%) of a light yellow solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (m, 2H), 6.95 (s, 1H), 6.71 (s, 2H), 6.68 (m, 2H), 4.54 (d, 1H, J = 13.6 Hz), 4.44 (d, 1H, J = 13.6 Hz), 3.84 (s, 6H), 3.84 (s, 3H), 3.05 (s, 6H); MS (DCI/NH_3) m/z 434 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-(methoxycarbonyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (10). To 100 mg (0.28 mmol) of the 4-(*N*,*N*-dimethylamino)benzoyl hydrazone derived from 3,4,5-trimethoxybenzaldehyde (prepared according to the general procedure used for hydrazones **5**) was added 2 mL of dimethyl pyrocarbonate. THF (1 mL) was added to improve solubility, and the mixture was heated in a sealed tube at 90 °C for 3 h. The tube was cooled with an ice bath and then opened. (*Caution: watch for gas evolution!*) The solvents were removed in vacuo, and the residue was triturated with a 1:1 mixture of diethyl ether and hexanes to produce a solid. The solid was washed further with diethyl ether to provide 75 mg (64% yield) of a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (s, 6H), 3.84 (s, 3H), 3.86 (s,3H), 3.87 (s, 6H), 6.66 (m, 2H), 6.69 (s, 2H), 6.92 (s, 2H), 7.80 (m, 2H), 7.99 (s, 1H); MS (ESI) m/z 416 $[M + H]^+$. Anal. Calcd for $C_{21}H_{25}N_3O_6$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.48; H, 5.65; N, 9.88.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-(acetoxyacetyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (11). To a solution of 100 mg (0.230 mmol) of chloroamide **9** in 1 mL of DMSO was added 50 mg (0.509 mmol) of potassium acetate. After 15 h, the reaction was poured into 20 mL of water and then extracted with ethyl acetate (3 × 5 mL). The combined ethyl acetate layers were back-extracted with water (1 × 5 mL) and brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give 95 mg (90%) of a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 3.05 (s, 6H), 3.83 (s, 3H), 3.85 (s, 6H), 5.05 (d, 1H, J = 12 Hz), 5.10 (d, 1H, J = 12 Hz), 6.69 (m, 2H), 6.70 (m, 2H), 6.93 (s, 1H), 7.73 (m, 2H); MS (DCI) m/z 458 $[M + H]^+$, 475 $[M + NH_4]^+$. Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.39; H, 5.95; N, 9.18. Found: C, 60.12; H, 5.87; N, 8.84.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-(azidoacetyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (12). To a suspension of 200 mg of chloroamide **9** in 1 mL of DMF was added 130 mg (2 mmol) of sodium azide. The mixture was stirred at ambient temperature for 15 h, then poured into 25 mL of 0.25 M $NaHCO_3$ solution, and extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate layers were back-extracted with water (1 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to give 203 mg (100%) of a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 3.84 (s, 3H), 3.85 (s, 6H), 4.26 (d, 1H, J = 12 Hz), 4.31 (d, 1H, J = 12 Hz), 6.70 (m, 2H), 6.71 (s, 2H), 6.95 (s, 1H), 7.73 (m, 2H); MS (DCI) m/z 441 $[M + H]^+$, 458 $[M + NH_4]^+$. Anal. Calcd for $C_{21}H_{24}N_6O_5$: C, 57.27; H, 5.49; N, 19.08. Found: C, 57.24; H, 5.53; N, 18.96.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-[(N,N-dimethylamino)acetyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (13). To 150 mg of chloroamide **9** were added 2 mL of 4.07 M dimethylamine in ethanol and 1 mL of DMF. The reaction was heated to a gentle reflux for 15 min, then poured into 25 mL of water, and extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. This was purified via silica gel chromatography, eluting with 95:5 EtOAc/MeOH, to give 40 mg (26%) of a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (s, 6H), 3.05 (s, 6H), 3.55 (d, 1H, J = 15 Hz), 3.68 (d, 1H, J = 15 Hz), 3.84 (s, 3H), 3.85 (s, 6H), 6.68 (m, 2H), 6.73 (s, 2H), 6.99 (s, 1H), 6.74 (m, 2H); MS (DCI) m/z 443 $[M + H]^+$. Anal. Calcd for $C_{23}H_{30}N_4O_5$: C, 62.57; H, 6.62; N, 12.69. Found: C, 62.28, H, 6.72, N, 12.62.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-(hydroxyacetyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (14). To 211 mg (0.461 mmol) of **11** were added 9 mL of methanol, 1 mL of water, and 300 mg (2.2 mmol) of potassium carbonate. The mixture was stirred at ambient temperature for 3 h, then diluted with 30 mL of water, and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to 180 mg (94%) of a white solid. The product could be purified by recrystallization from CH_3CN: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 3.84 (s, 3H), 3.85 (s, 6H), 4.53 (t, 2H, J = 4.6 Hz), 6.68 (m, 2H), 6.70 (s, 2H), 6.94 (s, 1H), 7.73 (m, 2H); MS (DCI) m/z 416 $[M + H]^+$, 433 $[M + NH_4]^+$. Anal. Calcd for $C_{21}H_{25}N_3O_6 \cdot 0.2CH_2Cl_2$: C, 58.88; H, 5.92; N, 9.72. Found: C, 58.80; H, 5.81; N, 9.50.

4-(Aminoacetyl)-2-[4-(*N*,*N*-dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (15). To a solution of 110 mg (0.250 mmol) of **12** in 2 mL of THF were added 66 mg (0.25 mmol) of triphenylphosphine and 3 drops of water. The mixture was stirred at ambient temperature for 16 h and then concentrated in vacuo to a white solid. The product amine was purified via silica gel chromatography, eluting with 8% CH_3OH in CH_2Cl_2, to give 40 mg (38%) of a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 3.84 (s, 3H), 3.84 (s, 2H), 3.85 (s, 6H), 6.68 (m, 2H), 6.71 (s, 2H), 6.92 (s, 1H), 7.74 (m, 2H); MS (DCI) m/z 415 $[M + H]^+$. Anal. Calcd for $C_{21}H_{26}N_4O_5 \cdot 0.25CH_2Cl_2$: C, 58.58; H, 6.13; N, 12.86. Found: C, 58.57; H, 6.23; N, 12.77.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-[(formylamino)-acetyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (16). To a mixture of 200 mg (0.483 mmol) of amine **15**, 111 mg (0.580 mmol) of EDCI, and 95 mg (0.580 mmol) of 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one were added 3 mL of DMF and then 40 μL (0.75 mmol) of 88% formic acid. The pH of the mixture was adjusted to 8 using triethylamine (15 drops), and then the mixture was stirred at ambient temperature for 2 h. The reaction was poured into 30 mL of 0.6 M $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were back-extracted with water (2 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to a white solid. The product was purified by recrystallization

from CH_3CN to give 101 mg (47%, one crop) of colorless crystals: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 3.84 (s, 3H), 3.85 (s, 6H), 4.51 (d, 2H, J = 5 Hz), 6.40 (br t, 1H), 6.70 (m, 2H), 6.69 (s, 2H), 6.91 (s, 1H), 7.75 (m, 2H), 8.28 (d, 1H, J = 1.4 Hz); MS (DCI) m/z 443 $[M + H]^+$. Anal. Calcd for $C_{22}H_{26}N_4O_6 \cdot 0.35H_2O$: C, 58.88; H, 6.00; N, 12.48. Found: C, 58.96; H, 6.16; N, 12.35.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-[(acetylamino)-acetyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxadiazoline (17). To a suspension of 51 mg (0.123 mmol) of amine **15** in 1 mL of DMF was added 2 drops of acetic anhydride. The suspension was stirred at ambient temperature for 1.5 h, during which time the starting material dissolved. The reaction was poured into 20 mL of 0.6 M aqueous $NaHCO_3$ solution and then extracted with CH_2Cl_2 (3 × 3 mL). The combined CH_2Cl_2 layers were back-extracted with water (2 × 3 mL) and brine (1 × 3 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to 56 mg (100%) of a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (s, 3H), 3.05 (s, 6H), 3.84 (s, 3H), 3.85 (s, 6H), 4.46 (t, 2H, J = 4.1 Hz), 6.26 (br t, 1H), 6.69 (m, 2H), 6.90 (s, 1H), 7.75 (m, 2H); MS (DCI) m/z 457 $[M + H]^+$, 474 $[M + NH_4]^+$. Anal. Calcd for $C_{23}H_{28}N_4O_6$: C, 60.52; H, 6.18; N, 12.27. Found: C, 60.53; H, 6.31; N, 12.14.

1-(1-Hydroxy-2-nitroethyl)-3,4,5-trimethoxybenzene (19). A solution of 3,4,5-trimethoxybenzaldehyde (10 g, 51 mmol) and nitromethane (10 mL) in ethanol at 0 °C was treated with 10% sodium hydroxide (21.4 mL, 53.5 mmol), stirred for 45 s, treated with 2% acetic acid (162 mL), stirred for 1 h in the ice bath, and filtered. The solid was washed with water and dried under vacuum to provide 9.0 g of the desired product as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (s, 2H), 5.42 (m, 1H), 4.61 (dd, 1H, J = 10.5, 14.4 Hz), 4.51 (dd, 1H, J = 3.6, 14.4 Hz), 3.85 (s, 3H), 3.89 (s, 6H), 2.88 (d, 1H, J = 4.5 Hz); MS (CDI/NH_3) m/z = 275 $[M + NH_3]^+$.

2-Amino-1-(3,4,5-trimethoxyphenyl)ethanol (20). Compound **19** was reduced with 10% Pd/C in acetic acid under 4 atm of H_2 for 3 h to provide amine **20** as its acetic acid salt: mp 103−104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H), 2.82−2.90 (m, 1H), 3.03−3.08 (m, 1H), 3.83 (s, 3H), 3.88 (s, 6H), 4.62−4.67 (m, 1H), 6.60 (s, 2H); MS (CDI/NH_3) m/z 228 $[M + H]^+$.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22a). To an ice-cooled solution of 287 mg (1.00 mmol, acetic acid salt) of amine **20** and diisopropylethylamine (2 mL) in dichloromethane (15 mL) was added slowly a solution of 4-(dimethylamino)benzoyl chloride (219 mg) in dichloromethane (10 mL). The reaction was stirred for 18 h, gradually warming to ambient temperature, and then concentrated to near dryness. The crude product was purified by silica gel chromatography, eluting with 1:1 hexanes/ethyl acetate, and loading in CH_2Cl_2 solution, to provide 340 mg (91%) of the desired amide as a white solid: mp 155−157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.03 (s, 6H), 3.44−3.57 (m, 1H), 3.83 (s, 9H), 4.02 (br s, 1H), 4.88−4.95 (m, 1H), 6.62 (s, 2H), 6.64 (d, 2H, J = 7 Hz), 7.66 (d, 2H, J = 7 Hz); MS (CDI/NH_3) m/z 375 $[M + H]^+$.

A solution of the amide (320 mg, 0.86 mmol) in chloroform (15 mL) at −20 °C was treated sequentially with pyridine (0.2 mL) and then triflic anhydride (0.2 mL), stirred for 30 min, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated. The concentrate was purified by flash chromotography on silica gel with 1:1 hexanes/ethyl acetate to provide 220 mg of oxazoline **22a** as an off-white solid:

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 3.85 (s, 9H), 3.96 (dd, 1H, J = 7 Hz), 4.43 (dd, 1H, J = 9 Hz), 5.57 (t, 1H, J = 7 Hz), 6.58 (s, 2H), 6.70 (d, 2H, J = 8 Hz), 7.93 (d, 2H, J = 8 Hz); MS (DCI/NH_3) m/z 357 $[M + H]^+$. Anal. Calcd. For $C_{20}H_{24}N_2O_4 \cdot 0.25H_2O$: C, 66.56; H, 6.84; N, 7.76. Found: C, 66.69; H, 6.88; N, 7.53.

2-(3-Amino-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22b). To a suspension of 3-amino-4-methoxybenzoic acid (1.00 g, 6.0 mmol) in acetone (15 mL) was added Fmoc-succinimide (2.63 g, 7.8 mmol) in acetone (15 mL), in small portions, alternating with the addition of 10% aqueous sodium carbonate solution, to keep the pH between 8 and 9. The resulting clear solution was stirred at ambient temperature overnight. The reaction was worked up by the addition of 3 N HCl. The precipitate was collected, washed with water and methylene chloride, and dried under vacuum to give the Fmoc-amino acid (1.54 g, 66%): ^{1}H NMR (d_6-DMSO) δ 8.91 (s, 1H), 8.23 (br s, 1H), 7.93 (d, 2H, J = 9.0 Hz), 7.7 (m, 3H), 7.45 (t, 2H, J = 7.0 Hz), 7.35 (t, 2H, J = 7.0 Hz), 7.14 (d, 1H, J = 9.0 Hz), 4.25−4.42 (m, 3H), 3.91 (s, 3H); MS (DCI/NH_3) m/z 407 $[M + NH_4]^+$.

To a stirred suspension of the Fmoc-amino acid (765 mg, 1.96 mmol) and ethanolamine (446 mg, 1.96 mmol) in tetrahydrofuran (20 mL) and DMF (1.5 mL) were added *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (747 mg, 1.96 mmol) and then *N*-methylmorpholine (0.22 mL). The reaction mixture was stirred at ambient temperature for 18 h. The solvent was removed on a rotary evaporator, and the residue was dissolved in methylene chloride, washed with water, dried over $MgSO_4$, and concentrated to give the ethanol amide (981 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 8.46 (br s, 1H), 7.80 (d, 2H, J = 9.0 Hz), 7.63 (m, 3H), 7.30−7.48 (m, 4H), 6.95 (d, 1H, J = 9.0 Hz), 6.63 (s, 3H), 6.60 (m, 1H), 4.90 (m, 1H), 4.51 (d, 2H, J = 9.0 Hz), 4.30 (t, 1H, J = 6.0 Hz), 3.84 (s, 9H), 3.82 (s, 3H); MS (ESI) m/z 621 $[M + Na]^+$.

A mixture of the ethanol amide (980 mg, 1.63 mmol) and [(methoxycarbonyl)sulfamoyl]triethylammonium hydroxide, inner salt (Burgess reagent) (507 mg, 2.13 mmol), in THF (25 mL) was heated at reflux for 2 h. After the THF was removed, the residue was dissolved in methylene chloride, washed with saturated sodium bicarbonate (2×) and then brine (1×), dried over $MgSO_4$, and concentrated to give the Fmoc-protected oxazoline (920 mg, 97%). To a stirred solution of the crude Fmoc-protected oxazoline (500 mg, 0.86 mmol) in acetonitrile (5 mL) was added diethylamine. The reaction was stirred at ambient temperature for 20 min before being concentrated on a rotary evaporator. The residue was purified by silica gel chromatography, eluting with methylene chloride/methanol/ammonium hydroxide (95:6:0.5), to provide oxazoline **22b** (227 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 2H), 6.81 (d, 1H, J = 9.0 Hz), 6.58 (s, 2H), 5.55 (m, 1H), 4.42 (m, 1H), 3.95 (m, 1H), 3.90 (s, 3H), 3.83 (s, 9H); MS (ESI) m/z 359 $[M + H]^+$. Anal. Calcd for $C_{19}H_{22}N_2O_5$: C, 63.68; H, 6.19; N, 7.82. Found: C, 63.92; H, 6.28; N, 7.81.

2-(3-Amino-4-methyphenyl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22c). This compound was prepared according to the same procedure as **22b**, substituting 3-amino-4-methylbenzoic acid (910 mg, 6.0 mmol) for 3-amino-4-methoxybenzoic acid. Purification via silica gel chromatography, eluting with CH_2Cl_2/methanol/ammonium hydroxide (95:5:0.7), gave 438 mg (45% overall yield) of **22c**: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 2H), 7.12 (d, 1H, J = 9.0 Hz), 6.55 (s, 2H), 5.53 (dd, 1H, J = 9.0, 12.0 Hz), 4.42 (dd, 1H, J = 12.0, 15.0 Hz), 3.95 (dd, 1H, J = 9.0, 15.0 Hz), 3.84 (s, 9H), 3.17 (br s, 2H); MS (ESI) m/z 343 $[M + H]^+$. Anal. Calcd for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.80; H, 6.53; N, 7.93.

2-(2-Oxobenzoxazol-6-yl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22d). This compound was prepared according to the same procedure as compound **22e**, substituting 2-oxobenzoxazole-6-carboxylic acid[15] for 1-methyl-1,2,3,4-tetrahydroquinoline-6-carboxylic acid. The product was purified by preparative TLC, eluting with ethyl acetate, to give 36 mg (50%) of oxazoline **22d**: ^{1}H NMR (d_6-DMSO, 300 MHz) δ 3.65 (s, 3H), 3.75 (s, 6H), 3.87 (dd, 1H, J = 8.1, 15.0 Hz), 4.38 (dd, 1H, J = 9.9, 15.0 Hz), 5.69 (dd, 1H, J = 8.1, 9.9 Hz), 6.67 (s, 2H), 7.19 (d, 1H, J = 8.4 Hz), 7.75 (d, 1H, J = 1.8 Hz), 7.77 (dd, 1H, J = 1.8, 8.4 Hz); MS (DCI) m/z 371 $[M + H]^+$. Anal. Calcd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56. Found: C, 61.34; H, 4.67; N, 7.56.

2-(*N*-Methyltetrahydroquinol-6-yl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22e). 1,2,3,4-Tetrahydroquinoline-6-carboxylic acid was prepared according to the published procedure.

[16] To a solution of 4.4 g (25 mmol) of 1,2,3,4-tetrahydroquinoline-6-carboxylic acid in 200 mL of methanol were added 10.3 g (75 mM) of K_2CO_3 and 7.7 mL (120 mmol) of iodomethane. The resulting mixture was stirred overnight at ambient temperature. The methanol was removed by concentration in vacuo. Water (200 mL) was added to the residual oil, and the mixture was extracted with ether (2 × 100 mL). The aqueous layer was acidified to pH 6 with 6 N HCl, and the mixture was extracted with ethyl acetate (2 × 200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.8 g (38%) of 1-methyl-1,2,3,4-tetrahydroquinoline-6-carboxylic acid as a yellow powder.

The acid (0.95 g, 5.0 mM) was coupled with amino alcohol **20** using EDCI, DMAP, and Et_3N in THF/DMF. Silica gel column chromatography (3:1 CH_2Cl_2/EtOAc and then 1:1 CH_2Cl_2/EtOAc) gave 100 mg (5%) of the amide as a yellow powder. To an ice-cooled solution of the amide (100 mg, 0.25 mM) in $CHCl_3$ (2 mL) was added thionyl chloride (36 μL, 0.50 mM), and the reaction was stirred for 30 min. Saturated aqueous $NaHCO_3$ (3 mL) was added, and the reaction was extracted with CH_2Cl_2 (3 × 4 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give a crude oil. Silica gel column chromatography (3:2 CH_2Cl_2/EtOAc) gave 15 mg (16%) of a light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89−7.81 (m, 1 H), 7.72 (br s, 1 H), 6.58−6.54 (m, 3H), 5.66−5.53 (m, 1H), 4.50−4.38 (m, 1H), 4.09−3.91 (m, 1H), 3.85 (s, 9H), 3.35 (t, 2H, J = 6.0 Hz), 2.98 (s, 3H), 2.78 (t, 2H, J = 6.0 Hz), 2.01−1.93 (m, 2H); MS (DCI/NH_3) m/z 383 $[M + H]^+$.

2-(1-Methyl-2,3-dihydroindol-5-yl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22f). A suspension of indole-5-carboxylic acid, *N*-[2-hydroxy-2-(3,4,5-trimethoxyphenyl)ethyl]-amide (207 mg, 0.56 mmol), and paraformaldehyde (168 mg, 5.6 mmol) in acetic acid (5 mL) was treated with sodium cyanoborohydride (176 mg, 2.79 mmol), stirred for 18 h, and then concentrated. The concentrate was partitioned between ethyl acetate and 10% $NaHCO_3$, washed sequentially with 10% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated to provide 218 mg of *N*-methylindoline-5-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (m, 2H), 6.63 (s, 2H), 6.38 (d, 1H, J = 8.1 Hz), 4.90 (m, 1H), 3.85 (m, 10H), 3.5 (m, 1H), 3.45 (t, 2H, J = 8.5 Hz), 3.14 (s, 1H), 2.99 (t, 2H, J = 8.5 Hz), 2.82 (s, 3H); MS (ESI) m/z 387 $[M + H]^+$.

Compound **22f** was prepared according to the same procedure as compound **22h**, substituting *N*-methylindoline-5-carboxylic acid for indole-5-carboxylic acid. The concentrate was purified by flash column chromatography on silica gel with 1:1 ethyl acetate/hexanes to provide 25 mg of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, 1H, J = 1.7, 8.5 Hz), 7.73 (d, 1H, J = 1.4 Hz), 6.58 (s, 2H), 6.38 (d, 2H, J = 8.5 Hz), 5.70 (app t, 1H, J = 9.2 Hz), 4.53 (dd, 1H, J = 10.2, 13.2 Hz), 4.22 (dd, 1H, J = 8.5, 12.9 Hz), 3.88 (s, 6H), 3.86 (s, 3H), 3.59 (t, 2H, J = 8.5 Hz), 3.06 (t, 2H, J = 8.5 Hz), 2.90 (s, 3H); MS (DCI/NH_3) m/z 369 $[M + H]^+$.

2-(1-Methylindol-5-yl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22g). To a solution of 1.00 g (6.20 mmol) of indole-6-carboxylic acid in 50 mL of DMF was added 745 mg of dry NaH, freshly prepared by washing 60% NaH in mineral oil with hexanes. The reaction was stirred for 25 min, and then 4 mL (62 mmol) of iodomethane was added. After being stirred for 18 h, the reaction was poured into 1.1 M $NaHSO_4$ and extracted with ethyl acetate. The organic layers were back-extracted sequentially with water, saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, filtered, and concentrated to give 1.14 g (97%) of 1-methyl-6-(methoxycarbonyl)-indole.

To a solution of 3.0 g (15.9 mmol) of 1-methyl-6-(methoxycarbonyl)indole in 50 mL of THF was added 50 mL of aqueous 1 M LiOH. The reaction was stirred at 50 °C for 24 h and then diluted with diethyl ether. The reaction was extracted twice with saturated aqueous $NaHCO_3$ solution, and then 6 M HCl was added to the combined aqueous layers to adjust the pH to 1. The acid layer was then extracted with ethyl acetate, and the combined ethyl acetate layers were dried over Na_2SO_4, filtered, and concentrated to give 2.65 g (95%) of 1-methylindole-6-carboxylic acid.

To a solution of 430 mg (2.46 mmol) of 1-methylindole-6-carboxylic acid in 20 mL of DMF were added 2 mL of triethylamine and 500 mg (3.69 mmol) of 1-hydroxybenzotriazole. Next, 519 mg (2.71 mmol) of EDCI was added, and then the mixture was stirred at ambient temperature for 15 min. After this time, 1.06 g (3.69 mmol) of amino alcohol **20** was added, along with 10 mg (catalytic amount) of 4-(*N*,*N*-dimethylamino)pyridine. After the resulting solution is stirred for 18 h, additional EDCI can be added if any carboxylic acid remains. The reaction was poured into ethyl acetate, then extracted once with aqueous 1 M $NaHSO_4$ solution, once with water, twice with saturated aqueous $NaHCO_3$ solution, and once with brine, dried over $MgSO_4$, filtered, and concentrated to give 471 mg (50%) of 1-methyl-1*H*-indole-5-carboxylic acid *N*-[2-hydroxy-2-(3,4,5-trimethoxyphenyl)ethyl]amide.

To a solution of 159 mg (0.41 mmol) of 1-methyl-1*H*-indole-5-carboxylic acid *N*-[2-hydroxy-2-(3,4,5-trimethoxyphenyl)ethyl]amide in 4 mL of THF was added 128 mg (0.54 mmol) of [(methoxycarbonyl)sulfamoyl]triethylammonium hydroxide, inner salt (Burgess reagent). The reaction was heated at reflux for 1 h, then cooled, and poured into saturated aqueous $NaHCO_3$ solution. The aqueous suspension was extracted with CH_2Cl_2, the organic layers were dried over Na_2SO_4, and the product was purified via silica gel chromatography, eluting with 70% ethyl acetate/hexanes, to give 78 mg (52%) of oxazoline **22g**: ^{1}H NMR (300 MHz, d_6-DMSO) δ 3.65 (s, 3H), 3.75 (s, 6H), 3.83 (s, 3H), 3.86 (dd, 1H, J = 8.4, 15.0 Hz), 4.37 (dd, 1H, J = 9.9, 15.0 Hz), 5.67 (dd, 1H, J = 8.4, 9.9 Hz), 6.55 (d, 1H, J = 3.3 Hz), 6.69 (s, 2H), 7.42 (d, 1H, J = 3.3 Hz), 7.53 (d, 1H, J = 8.7 Hz), 7.78 (dd, 1H, J = 1.5, 9.0 Hz), 8.16 (s, 1H); MS (DCI/NH_3) m/z 367 $[M + H]^+$. Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.57; H, 5.92; N, 7.48.

2-(Indol-5-yl)-5-(3,4,5-trimethoxyphenyl)-Δ2,3-oxazoline (22h). A solution of 5-indolecarboxylic acid (2.0 g, 12.4 mmol) in DMF (25 mL) at ambient temperature was treated portionwise with 1,1′-carbonyldiimidazole (2.1 g, 13.0 mmol). In a separate reaction, a suspension of amino alcohol **20** in DMF (20 mL) was treated with diisopropylethylamine (5.0 mL, 28.7 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (30.3 mg, 0.25 mmol). When the suspension of the amino alcohol cleared, the imidazolide solution was transferred dropwise to the amino alcohol solution. The pink solution was stirred at ambient temperature for 3 h, treated with water (100 mL), and adjusted to pH 5.5 with 3 N HCl to cause precipitation of a white solid. The solid was filtered, washed with cold water, and dried in a vacuum oven to provide 3.7 g of the desired amide as a white solid.

A solution of the above amide (200 mg, 0.54 mmol) in THF (10 mL) was treated with [(methoxycarbonyl)sulfamoyl]triethylammonium hydroxide, inner salt (Burgess reagent) (142 mg, 0.60 mmol), stirred at reflux for 1 h, cooled, and partitioned between ethyl acetate and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The concentrate was purified by flash column chromotography on silica gel with 1:1 hexanes/ethyl acetate to provide 120 mg of oxazoline **22h** as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 9H), 4.04 (dd, 1H, J = 8.7, 15.0 Hz), 4.50 (dd, 1H, J = 9.9, 15.0 Hz), 5.63 (dd, 1H, J = 8.7, 9.9 Hz), 6.61 (s, 2H), 6.64 (m, 1H), 7.28 (t, 1H, J = 3.0 Hz), 7.46 (d, 1H, J = 9.7 Hz), 7.95 (dd, 1H, J = 1.8, 9.7 Hz), 8.37 (s, 1H), 8.43 (br s, 1H); MS (DCI/NH_3) m/z 353 $[M + H]^+$. Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.42; H, 5.58; N, 7.77.

5-(3-Methyl-4-aminophenyl)-2-acetyl-3-(3,4,5-trimethoxyphenyl)-Δ1,5-pyrazoline (28). A mixture of 3-methyl-4-nitrobenzoic acid (**23**) (1.80 g, 0.01mol) and 1.1 equiv of 1,1′-carbonyldiimidazole was dissolved in THF. In a separate flask, 3,4,5-trimethoxyacetophenone (2.10 g, 0.01 mol) was dissolved in THF at −78 °C, 1.1 equiv of LiHMDS was added to the ketone solution, and the reaction was stirred for 1 h. The nitrobenzoic acid solution was added slowly at −78 °C, and the resulting mixture was warmed to ambient temperature

and stirred for 2 h. After aqueous workup, 1,3-diketone **24** was purified through silica gel column chromatography, eluting with hexane/EtOAc (3:2): yield 2.80 g (75%).

To a solution of 1,3-diketone **24** in CH_2Cl_2 and EtOH was added 1.1 equiv of hydrazine monohydrate, and then the solution was stirred at room temperature overnight. After aqueous workup, acetic anhydride was added and heated. After aqueous workup, the nitro group was hydrogenated on Pd/C (10%) in EtOH. The catalyst was filtered away, and the solvent was removed in vacuo to give regioisomeric amides **26** and **27**. The isomers were separated and purified by silica column chromatography. The slower moving compound was pyrazole **28**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.54 (s, 2H), 2.23 (s, 3H), 2.79 (s, 3H), 3.89 (s, 3H), 3.96 (s, 6H), 6.59 (s, 1H), 6.75 (d, 1H, J = 7.5 Hz), 7.10 (s, 2H), 7.18 (d, 1H, J = 7.5 Hz), 7.20 (s, 1H); MS (DCI/NH_3) m/z 382 $[M + H]^+$. Anal. Calcd for $C_{21}H_{23}N_3O_4 \cdot 0.25H_2O$: C, 65.36; H, 6.14; N, 10.89. Found: C, 65.58; H, 5.83; N, 10.76.

***N*-Boc-*N*′-[4-(*N*,*N*-dimethylamino)benzoyl]hydrazine (29).** **29** was prepared according to the general synthesis for hydrazides **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (m, 2H), 7.60 (m, 1H), 6.66 (m, 3H), 3.04 (s, 6H), 2.49 (s, 9H); MS (DCI/NH_3) m/z 280 $[M + H]^+$.

***N*-Boc-*N*′-[4-(*N*,*N*-dimethylamino)thiobenzoyl]hydrazine (30).** To a solution of 1.5 g (5.15 mmol) of **29** in 35 mL of THF was added 2.08 g (5.15 mmol) of 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent). The mixture was warmed to 45 °C, stirred for 3 h, then cooled, filtered to remove Lawesson's reagent, and concentrated under reduced pressure. The thiohydrazide was crystallized from hot absolute EtOH to give a yellow solid: yield 825 mg (54%).

To 700 mg (2.28 mmol) of the thio-Boc-hydrazide in 10 mL of CH_2Cl_2 was added 10 mL of trifluoroacetic acid. The solution was stirred for 2 h at ambient temperature, and then the solvents were evaporated under reduced pressure to give thiohydrazide **30** as its TFA salt.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-Δ2,3-thiadiazoline (32). To hydrazine **30** was added 447 mg (2.28 mmol) of 3,4,5-trimethoxybenzaldehyde dissolved in 20 mL of EtOH. This mixture turned red, and then a yellow solid precipitated after 30 min. This was collected and washed with EtOH to give the desired product **32** (677 mg, 80%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.05 (s, 6H), 3.92 (s, 3H), 3.95 (s, 6H), 6.75 (d, 2H, J = 12 Hz), 7.26 (s, 2H), 7.87 (d, 2H, J = 12 Hz); MS (DCI/NH_3) m/z 372 $[M + H]^+$. Anal. Calcd for $C_{19}H_{21}N_3O_3S \cdot 0.25H_2O$: C, 60.70; H, 5.76; N, 11.18. Found: C, 60.99; H, 5.81; N, 11.03.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-4-acetyl-5-(3,4,5-trimethoxyphenyl)-Δ2,3-thiadiazoline (33). A 300 mg (1.02 mmol) sample of thio-Boc-hydrazine **30** and 1 mmol of 3,4,5-trimethoxybenzaldehyde were dissolved in 5 mL of CH_2Cl_2 and 5 mL of trifluoroacetic acid and stirred for 2 h at ambient temperature, and then the solvents were evaporated under reduced pressure. Acetic acid (2 mL) was added, and the mixture was stirred for 20 min under argon. Acetic anhydride (4 mL) was added, and then the reaction was heated to 95 °C and stirred for 30 min. The solvents were evaporated, and then the residue was suspended in saturated aqueous $NaHCO_3$ and extracted with EtOAc. The product was purified via silica gel chromatography, eluting with hexane/EtOAc (4:1 to 3:2), and crystallized from ether to give 212 mg (50%) of compound **33**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.43 (s, 3H), 3.04 (s, 6H), 3.82 (s, 3H), 3.84 (s, 6H), 6.57 (s, 2H), 6.70 (d, 2H, J = 11 Hz), 6.98 (s, 1H), 7.62 (d, 2H, J = 11 Hz); MS (FAB) m/z 416 $[M + H]^+$. Anal. Calcd for $C_{21}H_{25}N_3O_4S$: C, 60.70; H, 6.06; N, 10.11. Found: C, 60.34; H, 5.97; N, 9.93.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)oxadiazole (34). To a suspension of 896 mg (5.00 mmol) of [4-(*N*,*N*-dimethylamino)benzoyl]hydrazine in 10 mL of ethyl acetate was added 0.8 mL of triethylamine, and then a solution of 1.15 g (5.00 mmol) of 3,4,5-trimethoxybenzoyl chloride in 5 mL of ethyl acetate was added. The mixture was stirred for 3 h at ambient temperature, then concentrated, suspended in ethanol, filtered, washed with water and then ethanol, and dried in vacuo to give diacylhydrazine **36**.

To 100 mg of diacylhydrazine **36** was added 1 mL of $POCl_3$. The reaction was stirred at ambient temperature for 24 h and then heated at 85 °C for 20 min. The mixture was poured over 20 mL of ice, then 25 mL of 2 M NaOH was added, and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. The product was recrystallized from toluene/hexanes to give 20 mg of a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (s, 6H), 3.93 (s, 3H), 3.98 (s, 6H), 6.80 (d, 2H, J = 9.2 Hz), 7.34 (s, 2H), 7.99 (d, 2H, J = 8.8 Hz); MS (DCI) m/z 356 $[M + H]^+$. Anal. Calcd for $C_{19}H_{21}N_3O_4 \cdot 0.05H_3PO_4$: C, 63.34; H, 5.92; N, 11.66. Found: C, 63.36; H, 5.79; N, 11.49.

2-[4-(*N*,*N*-Dimethylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-1,3-dioxolane (37). To 1.07 g (5.51 mmol) of 3,4,5-trimethoxystyrene[9] in 3 mL of acetone and 968 mg (8.27 mg) of *N*-morpholine *N*-oxide in 1 mL of water was added 1 mL of a 0.02 M solution of OsO_4 in *t*-BuOH. The mixture was stirred at ambient temperature for 71 h, and then 1.5 g of $Na_2S_2O_5$ was added. After 1 h, the reaction was filtered, the salts were washed with acetone, and the solvents were removed in vacuo. The brown residue was taken up in 10 mL of ethyl acetate and extracted with 1 M H_2SO_4 (2 × 5 mL), and then the combined aqueous layers were back-extracted with ethyl acetate (2 × 10 mL). The combined ethyl acetate layers were washed with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to give 1.22 g of a brown oil.

To a solution of 1.22 g (5.5 mmol) of the crude 1,2-diol, 900 mg (6.0 mmol) of 4-(*N*,*N*-dimethylamino)benzaldehyde, and 1.4 g (7.4 mmol) of *p*-toluenesulfonic acid in 5 mL of toluene was added 1.2 g of crushed 4 Å molecular sieves. The reaction was stirred at ambient temperature for 3 h, and then 10 mL of saturated aqueous $NaHCO_3$ solution was added. The mixture was filtered, and then the layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 5 mL), and then the combined organic layers were washed with water (1 × 5 mL) and then brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. This was purified via silica gel chromatography, eluting with 30% ethyl acetate/hexanes, to give the product as a mixture of acetal diastereomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.09 (s, 6H), 3.84 (s, 3H major), 3.84 (s, 6H major), 3.86 (s, 3H minor), 3.88 (s, 6H, minor), 3.96 (dd, 1H major, J = 5.9, 8.0 Hz), 4.33 (dd, 1H major, J = 7.1, 7.8 Hz), 4.50 (m, 1H minor), 5.11 (dd, 1H major, J = 6.6, 7.0 Hz), 5.93 (s, 1H major), 6.12 (s, 1H major), 6.63 (s, 2H minor), 6.65 (s, 2H major), 6.74 (m, 2H major), 7.41 (m, 2H minor), 7.44 (m, 2H major); MS (DCI/NH_3) m/z 360 $[M + H]^+$. Anal. Calcd for $C_{20}H_{25}NO_5$: C, 66.84; H, 7.01; N, 3.90. Found: C, 66.94; H, 7.14; N, 3.89.

In Vitro Assays. Cells in 48- or 96-well plates in growth medium were treated with compounds at different concentrations for 48 h. The cells were trypsinized, and the number of cells in each well was determined using a Coulter counter.[8]

In Vivo Studies. Mice were obtained from Charles River, Wilmington, MA. Female BALB/cAnNCrlBR × male DBA/2NCrlBR (hereafter referred to as CDF1), female C57BL/6NcrlBR × male DBA/2NCrlBR (hereafter referred to as BDF1), and C57BL/6 mice were housed 10 animals/cage on bedding and given free access to food and water. P388 leukemia cells were obtained from Arthur D. Little, Inc., while B16 melanoma and M5076 reticulum cell sarcoma were obtained from the National Cancer Institute (Bethesda, MD). For iv inoculations, P388, B16, and M5076 tumor cells were harvested from ascites fluid of previously inoculated mice. P388 (10^6 cells/mouse) was injected iv into CDF1 mice, and M5076 and B16 (10^6 cells/mouse) were injected iv into BDF1 mice. The drug efficacy of iv tumors was based on the percent ILS of treated versus untreated mice.

For the solid tumor model, M5076 cells were prepared from homogenization of the solid tumor tissue. A 1:10 (w/v) tumor homogenate using Hanks buffered salt solution was made, and 0.5 mL was injected sc into C57BL/6 mice. The tumors were

measured with vernier calipers. The drug efficacy was based on the percent ILS using the mean percent increase in life span compared to the vehicle in time for a 1 cm^3 tumor. For all models, group sizes consisted of 10 mice for each treatment and all experiments began on day 1 post tumor inoculation.

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